Remarks

Entry of the foregoing, reexamination, and further and favorable reconsideration of the subject application in light of the following remarks and pursuant to 37 C.F.R. §1.112, are respectfully requested. Claims 21-34 were previously pending in this application. Claim 35 has been cancelled. Claims 22-24, 26-27, 32, 34, and 38 are amended. Claims 21-34 and 36 through 39 are currently under consideration.

§ 112 Rejections

Claims 21-39 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner found that "Applicant broadly claims an isolated nucleic acid molecule comprising a nucleic acid sequence encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase found in Arabidopsis; an isolated polynucleotide comprising SEQ ID NO: 1; an isolated polynucleotide comprising a nucleotide sequence having at least 70% to 95% sequence identity to SEQ ID NO: 1; and a polynucleotide that hybridizes to SEQ ID NO: 1 under conditions of unspecified stringency." *(Office Action at 2)* The Examiner found that "Applicant teaches an isolated polynucleotide of SEQ ID NO: 1 encoding the polypeptide of SEQ ID NO: 2" and that "Applicant does not teach any other isolated polynucleotide other than SEQ ID NO: 1 encoding the polypeptide of SEQ ID NO: 2 or any other polynucleotides hybridizing thereto. Given the claim breadth and lack of guidance as discussed above, the specification does not provide an adequate written description of the claimed invention." *(Office Action at 2)* Further, the Examiner found that "methods of its use are also inadequately described". *(Office Action at 2)* Applicants respectfully disagree.

Applicants thank the Examiner for the acknowledgement that the specification describes an isolated polynucleotide of SEQ ID NO: 1 encoding the polypeptide of SEQ ID NO: 2, but respectfully disagree with the remainder of Examiner's conclusions.

An adequate written description of a genus of nucleic acids may be achieved by either "a recitation of a representative number of [nucleic acid molecules], defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to members of the genus." *Regents of the University of California v. Eli Lilly and Co.*, 119 F.3d 1559, 1568-69 (Fed. Cir. 1997). This applicants have done.

For example, applicants have disclosed the genomic nucleic acid sequence encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase found in *Arabidopsis*, SEQ ID NO: 1. Applicants have also provided the deduced amino acid sequence, SEQ ID NO: 2. Further, applicants have described the cloning of a cDNA clone encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *Arabidopsis*, see specification at Example

4, pages 27-29. One of ordinary skill in the art, provided with the teachings of the present application, would conclude that applicants were indeed in possession of the cDNA sequence as well as the genomic sequence. In addition, applicants have deposited plasmid pDXR-AT, referenced at page 28 of the specification in Example 4, with the American Type Culture Collection (see Declaration of Henry E. Valentin, executed October 8, 2002), as deposit number PTA4727 (Attachment 1). This plasmid contains the cDNA clone encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *Arabidopsis* and the sequence can be readily determined by one of ordinary skill in the art.

As stated above, applicants respectfully disagree with the Examiner's assessment that the claims encompass "a polynucleotide that hybridizes to SEQ ID NO: 1 under conditions of unspecified stringency." *(Office Action at 2)*

Part (g) of each of Claims 21-35 are worded identically. All include the phrase "that hybridize under stringent conditions". Thus, the Examiner is incorrect in concluding that the claims contain "conditions of unspecified stringency". Further, the specification defines "stringent conditions" at page 8, lines 22-30 through page 9, line 2 of the specification:

> As used herein, the terms "stringent conditions" and "stringent hybridization conditions" mean that hybridization will generally occur if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at approximately 65°C. Other hybridization and wash conditions are well known and are exemplified in Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly Chapter 11.

Further description for the rejected claims can be found in the specification, in at least the following locations: for an isolated polynucleotide comprising a nucleotide sequence having at least 70% to 95% sequence identity to SEQ ID NO: 1, see the specification at pp. 8, 10-12.

Applicants respectfully submit that the rejections under 35 U.S.C. § 112, first paragraph, should be withdrawn.

Claims 21-39 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner maintains that "applicant broadly claims methods for increasing disease resistance in a plant, for increasing or decreasing isoprenoid content in a plant using constructs comprising DNA in either sense or antisense orientation, for

increasing the non-mevalonate isoprenoid biosynthetic flux in plants transformed with an isolated polynucleotide comprising SEQ ID NO: 1, an isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide of SEQ ID NO: 2, and isolated polynucleotide comprising a nucleotide sequence having at least 70% to 95% sequence identity to SEQ ID NO: 1, or a polynucleotide that hybridizes to SEQ ID NO: 1 under conditions of unspecified stringency". *(Office Action at 3-4)* The Examiner found that "Applicant teaches isolation of SEQ ID NO; 1 encoding SEQ ID NO: 2 from *Arabidopsis* by searching an EST database using an *E. coli* clone encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase, RACE PCR of the 5' ends and sequencing of the completed EST clones. Applicant does not teach isolation of any other sequence encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase other than SEQ ID NO: 1 and does not teach any method of using the isolated polynucleotide of SEQ ID NO: 1 from *Arabidopsis*." *(Office Action at 4)*

Further, the Examiner states that the phenotypic character expected from expression of a DNA construct often cannot be reliably predicted, citing Smith *et al.* for the finding that the antisense expression of a polygalacturonase gene in transgenic tomato had no effect on fruit ripening. Still further, the Examiner finds that isolating DNA fragments using stringent hybridization conditions does not always select for DNA fragments whose contiguous nucleotide sequence is the same or nearly the same as the probe, citing Fourgoux-Nicol *et al.* and also that the isolation or manufacture of DNA sequences with a degree of identity to a target sequence introduces an element of unpredictability. *(Office Action at 4-5)* According to the Examiner, the limitation is introduced in finding regions that would adequately enable either PCR amplification or southern hybridization and would entail using either degenerate primers or probes with limited homology and in recognizing conserved regions of class of polynucleotides encoding polypeptides with a common specific activity, thus isolating many genes other than those of interest. The Examiner cites Broun *et al.* as an example of the inherent unpredictability in isolation of a sequence with similar sequence identity encoding the same protein activity. *(Office Action at 5)* The Examiner additionally states that "evidence for a non-limiting role for reductoisomerases in the non-mevalonic isoprenoid biosynthetic pathway in bacteria and plants suggests that altering the expression using antisense or sense or a reductoisomerase encoding gene would have no effect, particularly since the enzyme just upstream of the reductoisomerase in the mevalonic isoprenoid biosynthetic pathway has been shown to be limiting for isoprenoid flux," citing Estevez *et al.* and Rodriguez *et al. (Office Action at 5-6)* The Examiner also finds that the likelihood of enhancing disease resistance from transformation with a gene involved in disease resistance cannot be predicted. *(Office Action at 6)* Applicants respectfully traverse the rejection.

More specifically, applicants respectfully disagree with the Examiner's assertion that "Applicant does not teach isolation of any other sequence encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase other than

SEQ ID NO: 1 and does not teach any method of using the isolated polynucleotide of SEQ ID NO: 1 from *Arabidopsis*." *(Office Action at 4)* For example, as set forth in more detail above, applicants have taught the genomic nucleic acid sequence encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase found in *Arabidopsis*, SEQ ID NO: 1, the deduced amino acid sequence, SEQ ID NO: 2, and the cloning of a cDNA clone encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *Arabidopsis*.

Methods of using the claimed invention are taught in the specification in at least the following locations: p. 4, first paragraph (modifying the isoprenoid content of host cells) and fifth paragraph (altering the isoprenoid levels and/or modulating ratios in host cells, modulation of isoprenoid content in host plant cells), p. 6, second full paragraph (constructs and methods for the production of altered expression of dxr in host cells, as well as methods for the modification of the isoprenoid pathway), p. 9, second full paragraph through p. 10, first full paragraph (hybridization probes), pp. 14 through 23 (constructs to direct the transcription or transcription and translation of the dxr sequences in a host cell, including, but not limited to plant cells), p. 15, last paragraph (discussing methods for inhibition of expression, including antisense), and p. 16, fourth paragraph (methods for altering the flux through the isoprenoid pathway).

Applicants also respectfully disagree with the Examiner's assessment that the claims encompass "a polynucleotide that hybridizes to SEQ ID NO: 1 under conditions of unspecified stringency."

Part (g) of each of Claims 21-35 are worded identically. All include the phrase "that hybridize under stringent conditions". Thus, the Examiner is incorrect in concluding that the claims contain "conditions of unspecified stringency". Further, the specification defines "stringent conditions at page 8, lines 22-30 through page 9, line 2 of the specification:

> As used herein, the terms "stringent conditions" and "stringent hybridization conditions" mean that hybridization will generally occur if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at approximately 65°C. Other hybridization and wash conditions are well known and are exemplified in Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly Chapter 11.

The Examiner relies on Smith, *et al.*, Broun *et al.*, and Fourgoux-Nicol *et al.*, apparently suggesting that those references teach unpredictability in the art of the present invention. *(Office Action at 4-5)* The Examiner, however, fails to acknowledge the teachings set forth in the specification. Subparts (c) through (g) of independent claims 22-24, 26-27, 32 and 34 as well as claim 38 all include the limitation "encoding 1-deoxy-D-xylulose 5-phosphate reductoisomerase". Thus, it is not just any sequence having specified similarity to SEQ

ID NO: 1 which is claimed. The sequence must also encode a 1-deoxy-D-xylulose 5-phosphate reductoisomerase. Confirming the specificity and activity of the proteins encoded by the nucleotides of the present invention as dxr enzymes is taught in the specification at, for example, p. 18, third and fourth paragraphs and Example 6.

Examiner's reliance upon Estevez *et al* and Rodriguez *et al.*, both from 2001, to assert a non-limiting role for reductoisomerases in the non-mevalonic isoprenoid biosynthetic pathway in bacteria and plants is also mistaken. The Examiner's attention is respectfully directed to Rodriguez-Concepcion *et al.* November 2002, Vol. 130 pp. 1079-1089, at 1085, col. 1 (Attachment 2). The authors indicate that "to date, DXS is the only enzyme of the MEP pathway that has been shown to have a limiting role for isoprenoid synthesis in all the systems analyzed...*the role of DXR is less clear.*" (emphasis added). The authors note the contrast between bacteria (overexpression studies suggest that DXR activity is not limiting for isoprenoid biosynthesis in bacteria) and peppermint (overexpression of DXR in peppermint led to increased isoprenoid synthesis). Further, the authors note that DXS expression predates that of DXR in some organs, such as developing inflorescences, suggesting that DXR instead of DXS might be limiting for the onset of plastid isoprenoid biosynthesis. *"Together, the results support a general regulatory role for DXS in controlling the metabolic flux through the MEP pathway, whereas DXR activity may be limiting or not depending on the species, organ and/or developmental stage."*

Thus, the Examiner's position that there is a non-limiting role for reductoisomerases in the non-mevalonic isoprenoid biosynthetic pathway in plants is incorrect. In addition, even if the Examiner's assertion of the non-limiting role for such reductoisomerases were correct, the overexpression of DXR in peppermint led to increased isoprenoid synthesis. See Mahmoud *et al.*, PNAS, 98(15):8915-8920, 17 Jul 2001 (Attachment 3).

For the purposes of facilitating prosecution, applicants have cancelled claim 35, without prejudice.

Applicants request that the § 112, first paragraph rejection be withdrawn.

§ 102 Rejections

Claims 21-24 and 36 through 39 stand rejected under 35 USC § 102(a) as being "anticipated by Sato *et al.* (GenBank Accession number AB009053 submitted November 27, 1999 and described in attached sequence report)." *(Office Action at 7)* The Examiner states that "Sato teaches the open reading frames of an Arabidopsis genomic clone comprising SEQ ID NO: 1 encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase enzyme and inherently teaches a construct and host cell comprising said DNA. Thus the reference teaches all the limitations of the Claims 21-24." *(Office Action at 7)* Applicants respectfully traverse the rejection.

Applicants have examined the Sato document provided by Examiner and respectfully submit that the Examiner is in error. Applicants submit that the GenBank history data from the GenBank web-site (NCBI.nlm.nih,gov) shows that the locus AB009053 appeared in GenBank in April 1998, based upon a November 27, 1997 submission by Nakamura (of the Sato group). This November, 1997 GenBank submission simply recites the entire sequence of clone MQB2 from chromosome 5 of *Arabidopsis*. No coding sequences for any gene were provided. Attached is a printout of the text file of the 1997 submission: apr98.txt (Attachment 4).

Again, information from the web-site shows that in August of 2000, GenBank was updated with the coding sequences for genes within the locus AB009053. In that update, the protein ID BAB 1084.1 is identified as dxr. Attached is a printout of the text file of the August 2000 update to AB009053 (Attachment 5).

The present application claims the benefit of provisional applications filed April 15, 1999 and July 30, 1999, and claims priority to an application filed on April 14, 2000. No coding sequences for any gene were provided in the AB009053 GenBank submission prior to any of those dates. Thus, Applicants respectfully request the withdrawal of the § 102(a) rejection based upon Sato.[1]

Claims 22-35 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Burkhardt *et al.* The Examiner maintains that "the claims are broadly drawn to an isolated polynucleotide encoding an unspecified protein in part (g) of claims that recite 'an isolated polynucleotide that hybridizes' to SEQ ID NO: 1 under conditions of unspecified stringency and duration; constructs, plant cells, and plants comprising said DNA that produce an isoprenoid compound of interest having altered or increased isoprenoid biosynthetic pathway activity. Burkhardt teaches increased phytoene production in rice transformed with a DNA construct comprising phytoene synthase from daffodil. *(Office Action at 7)* Thus, the reference teaches all the limitations of Claims 22-35." Applicants respectfully traverse the rejection.

Applicants respectfully submit that the Examiner's application of Burkhardt *et al.* is incorrect. Whatever else it may teach, Burkhardt *et al.* does not teach a sequence which has the activity of 1-deoxy-D-xylulose-5-phosphate. Further, as previously explained, the claims contain hybridization conditions. Part (g) of each of Claims 21-35 are worded identically. All include the phrase "that hybridize under stringent conditions". The specification defines "stringent conditions at page 8, lines 22-30 through page 9, line 2 of the specification:

> As used herein, the terms "stringent conditions" and "stringent hybridization conditions" mean that hybridization will generally occur if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution

[1] GenBank was updated in June, 2000 for the locus AF148852 definition arabidopsis thaliana 1-deoxy-D-xylulose-5-phosphate. Attached is a printout of the text file of the 1999 submission for AF148852 (Attachment 6).

comprising 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at approximately 65°C. Other hybridization and wash conditions are well known and are exemplified in Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly Chapter 11.

Applicants respectfully submit that the Examiner has not met his burden of proving that claims 21-35 are anticipated by Burkhardt *et al.* and further, that the rejection should be withdrawn.

§ 103 Rejections

Claims 21-39 stand rejected under 35 U.S.C. § 103 (a) as being unpatentable over Croteau *et al.* in view of Sato *et al.* The Examiner describes Croteau *et al.* as teaching an isolated DNA sequence encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase enzyme from *Mentha x piperita* for use in enhancing the production of chlorophyll, terpenoids, phytoalexins, and carotenoids in a plant, and antisense suppression of 1-deoxy-D-xylulose 5-phosphate reductoisomerase activity. *(Office Action at 8-9)* The Examiner states that Croteau *et al.* does not teach the *Arabidopsis* polynucleotide sequence of SEQ ID NO: 1 encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase enzyme of SEQ ID NO: 2. The Examiner relies upon the teachings of Sato *et al.* as set forth earlier. The Examiner concludes that it would have been obvious at the time of Applicant's invention to modify the invention of Croteau *et al.* to include the *Arabidopsis* 1-deoxy-D-xylulose 5-phosphate reductoisomerase sequence taught by Sato *et al. (Office Action at 9)* The Examiner identifies the motivation to modify the invention of Croteau *et al.* as " the knowledge common in the art that 1-deoxy-D-xylulose 5-phosphate reductoisomerase enzyme plant genes are valuable materials for genetic engineering of plants to overproduce or restrict the production of carotenoids, terpenoids, chlorophyll, and phytoalexins using the methods taught by Croteau", *(Office Action at 9)* and further finds that "one would have had a reasonable expectation of success of expressing genes in transformed plants and plant cells. Modulated disease resistance would have been an inherent property of the transformed plants, either due to the transgene or to its insertion in a gene conferring disease resistance." *(Office Action at 9)* Applicants respectfully traverse this rejection.

Applicants have demonstrated above that the Examiner's reliance upon Sato *et al.* is mistaken. As set forth above, Sato *et al.* does not teach an *Arabidopsis* 1-deoxy-D-xylulose 5-phosphate reductoisomerase sequence. Therefore, assuming solely for the sake of responding to this Office Action that the Examiner's characterization of Croteau *et al.* is correct, there is no motivation for combining the invention of Croteau *et al.* with the teaching of Sato *et al.*, and even if such motivation were present, the combination does not suggest the invention of claims 21-39.

Withdrawal of rejections under 35 U.S.C. § 103 is respectfully requested.

Conclusion

The presently pending claims are believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue. The Examiner is respectfully requested to contact applicants' undersigned representative at 847 457 5055 to address any unresolved issues remaining in this application.

In the event that extensions of time beyond those petitioned for herewith are necessary to prevent abandonment of this patent application, then such extensions of time are hereby petitioned. Applicants do not believe that any additional fees are due in conjunction with this filing. However, if any fees under 37 C.F. R. § 1.16 or § 1.17 are required in the present application, including any fees for extensions of time, then the Commissioner is hereby authorized to charge such fees to the Renessen LLC Deposit Account No. 50-1100, referencing docket number REN 01-020-Con.

Respectfully submitted,

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